Coupling mixture reference models with DGT-perceived metal flux for deciphering the nonadditive effects of rare earth mixtures to wheat in soils

Gong, Bing; He, Erkai; Peijnenburg, Willie J.G.M.; Iwasaki, Yuichi; Van Gestel, Cornelis A.M.; Cao, Xinde; Zhao, Ling; Xu, Xiaoyun; Qiu, Hao

published in
Environmental Research
2020

DOI (link to publisher)
10.1016/j.envres.2020.109736

document version
Publisher's PDF, also known as Version of record

Environmental Research 188 (2020) 109736

Contents lists available at ScienceDirect

Environmental Research

journal homepage: www.elsevier.com/locate/envres

Coupling mixture reference models with DGT-perceived metal flux for deciphering the nonadditive effects of rare earth mixtures to wheat in soils

Bing Gong[a], Erkai He[b], Willie J.G.M. Peijnenburg[c,d], Yuichi Iwasaki[e], Cornelis A.M. Van Gestel[f], Xinde Cao[a], Ling Zhao[a], Xiaoyun Xu[a], Hao Qiu[a,b,*]

[a] School of Environmental Science and Engineering, Shanghai Jiao Tong University, Shanghai, 200240, China
[b] Guangdong Provincial Key Laboratory of Environmental Pollution Control and Remediation Technology, Sun Yat-sen University, Guangzhou, 510275, China
[c] Institute of Environmental Sciences, Leiden University, Leiden, 2333CC, the Netherlands
[d] National Institute of Public Health and the Environment, Center for the Safety of Substances and Products, Bilthoven, 3720 BA, the Netherlands
[e] Research Institute of Science for Safety and Sustainability, National Institute of Advanced Industrial Science and Technology, 16-1 Onogawa, Tsukuba, 305-8569, Japan
[f] Department of Ecological Science, Faculty of Science, Vrije Universiteit, De Boelelaan 1085, 1081, HV, Amsterdam, the Netherlands

ARTICLE INFO

Keywords:
Bioavailability
Toxicity
Rare earth
Mixture
Triticum aestivum

ABSTRACT

The risk assessment of mixtures of rare earth elements (REEs) is hampered by a lack of fundamental understanding of their interactions in different soil types. Here, we assessed mixture interactions and toxicity to *Triticum aestivum* of Y and Ce in four different soils in relation to their bioavailability. Mixture toxicity was modelled by concentration addition (CA) and independent action (IA), in combination with different expressions of exposure: three equilibrium-based doses (total soil concentrations $[M]_{tot}$, free ion activity in soil solution $\{M^{3+}\}$, and the fraction (f) of metal ions bound to the biotic ligands (BLs)) and one kinetically controlled dose ($[M]_{flux}$) metrics. Upon single exposure, REE toxicity was increasingly better described when using exposure expressions based on deepened understanding of their bioavailability: $[M]_{flux} > f > \{M^{3+}\} > [M]_{tot}$. The mixture analyses based on $[M]_{tot}$ and $\{M^{3+}\}$ displayed deviations from additivity depending on the soil type. With the parameters derived from single exposures, the BLM approach gave better predictions of mixture toxicity ($R^2 \sim 0.70$) than when using CA and IA based on either $[M]_{tot}$ or $\{M^{3+}\}$ ($R^2 < 0.64$). About 30% of the variance in toxicity remained unexplained, challenging the view that the free metal ion is the main bioavailable form under the BLM framework based on thermodynamic equilibrium. Toxicity was best described when accounting for changes in the size of the labile metal pool by using a kinetically controlled dose metric ($R^2 \sim 0.80$). This suggests that dynamic bioavailability analysis could provide a robust basis for modeling and reconciling the interplays and toxicity of metal mixtures in different soils.

1. Introduction

Soil pollution has become one of the primary constraints for the sustainable development of economy and society with the potential to destroy the function of soil ecosystems and threaten the quality of agricultural products (Amundson et al., 2015; Wall et al., 2015). Soils contaminated with rare earth elements (REEs) have become of great concern (Adeel et al., 2019; Dinali et al., 2019; Gui et al., 2017; Mihajlovic and Rinklebe, 2018). REEs act as protagonists in modern-day tech industries and common commodities in daily life (Zaimes et al., 2015). The rapidly growth of global demand for REEs has motivated an increasing number of mining activities, causing substantial release of REEs into the environment (Sprecher et al., 2015). It was for instance found that concentrations of REEs in soils near REE mining sites in China can exceed their contents in the earth's crust by about a factor 200, which might pose threats to food supply and human health (Liang et al., 2014). However, relevant regulatory threshold values and environmental quality standards regarding REEs have not yet been set.

Soil contamination with mixtures of REEs are more ubiquitous compared to single toxicant pollution (Romero-Freire et al., 2018; Tyler, 2004). Current risk evaluation of chemicals in soil is, however, almost always based on the effects of single metals, which can be justified only if the exposure to mixtures does not bear the risk of an increased toxicity relative to the toxicity upon single exposure (Backhaus and Faust, 2012). In the natural environment, the occurrence of more-than-additive (synergism) or less-than-additive (antagonism) effects is nothing new now among multiple metals since they may interact with each other (Drakvik et al., 2020; Norwood et al., 2003). In case of REEs

* Corresponding author. School of Environmental Science and Engineering, Shanghai Jiao Tong University, Shanghai, 200240, China.
E-mail address: haoqiu@sjtu.edu.cn (H. Qiu).

https://doi.org/10.1016/j.envres.2020.109736
Received 25 April 2020; Received in revised form 20 May 2020; Accepted 24 May 2020
Available online 02 June 2020
0013-9351/

mixtures, no clear pattern of deviation from the additivity has appeared to date under hydroponic conditions, not to mention in soils (Gong et al., 2018). This might be due to specific and non-specific interactions at various levels (e.g., physicochemical, physiological, and toxicological interactions) (Komjarova and Blust, 2009; Van Ginneken et al., 2015). Therefore, there is a need to develop appropriate approaches for modeling and reconciling the interplays and toxicity of mixtures of REEs in soils.

In general, two basic concepts can be used to model mixture toxicity: concentration-addition (CA) and independent action (IA) (Backhaus and Faust, 2012). Ideally, if the components in the mixture own equal modes of actions (MOAs), CA is suitable for predicting the combined effects (Gopalapillai and Hale, 2017). In contrast to the CA-concept, IA is often chosen when the components act dissimilarly (Qiu and He, 2017). Our previous study on modeling the toxicity of a mixture of REEs in hydroponic cultures showed inconsistent patterns (additivity, antagonism, or synergy) of REE mixture toxicity based on different dose metrics (e.g., total dissolved metal concentrations and free metal activities) (Gong et al., 2018). As a consequence, in the more complex soil environment the applicability of the additivity-based models for REE mixtures remains obscure.

Understanding bioavailability of REEs is a key step to analyze their interplays and mixture toxicity across different soil types. Previous publications on terrestrial systems have reported that the use of CA or IA models based on total metal concentrations failed to normalize variations of metal toxicity in different soils (Lofts et al., 2004). Later, the free metal ion activity in soil solution was accepted as a more reliable predictor of toxicity than the total metal concentration by including considerations of bioavailability (Campbell, 1995; Thakali et al., 2006; Morel, 1983). According to the biotic ligand model (BLM), metal toxicity depends mainly on the free metal ion activity in solution, which can be affected not only by competition but also by complexation (De Schamphelaere and Janssen, 2002). For trivalent REEs, the applicability of the BLM theory for quantifying the toxicity of individual elements has been verified in hydroponic cultures (Gong et al., 2019). Thus, it seems feasible to develop an extended BLM considering bioavailability and mixture interactions for modeling and predicting the toxicity of mixtures of REEs in soils. If there are competitive effects between different elements for binding to the same biotic ligands (BLs), the total amount of REEs bound to those BLs can be used to predict their combined toxicity using a BLM-based CA approach. Alternatively, if no competitive binding effect occurs, the estimations derived from individual REE exposures can be incorporated into a BLM-based IA approach. The BLM theory has shown its applicability to aquatic metal mixture toxicity (Cremazy et al., 2019; Di Toro et al., 2001; Hatano and Shoji, 2008; Jho et al., 2011; Playle, 2004). However, only limited attempts have been done to extend the BLM framework to explain mixture effects in soils (Qiu et al. 2015, 2016). As far as we know, no attempt has been done to extend the BLM to consider REE mixture scenarios in different soils.

Like the aquatic BLM, the terrestrial BLM is based on the assumption of equilibrium partitioning. This assumption may not be valid when the sorption reactions with the reactive solid phases are slow relative to the internal transport of elements into organisms and the subsequent expression of the biological response. Equilibrium may also not be assumed when the metal transport into the organism is limited by diffusional control across the (static) boundary layer around the interfacial cells. Both kinetic control and diffusional limitations have been shown to occur in metal-deficient soils (Luo et al. 2010, 2014). Under such circumstances, the kinetically labile metal, assessed by for example the time-resolved device of Diffusive Gradients in Thin films (DGT), is assumed to represent metal bioavailability much better (Luo et al., 2014). In soil solution, the kinetically labile metal pool includes not only the free metal ion but also labile metal complexes (Ernstberger et al., 2005). Metal captured by DGT may accurately mimic the dynamic reaction processes in soils by locally depleting the metal concentration in soil solution and by inducing resupply of metals during different processes (e.g., diffusional transport, release of labile metal from the solid phase, and resupply from the solid phase to solution) (Ernstberger et al., 2005; Luo et al., 2014). The technique of DGT has been successfully applied to evaluate the bioavailability of single metals (Ernstberger et al., 2005; Luo et al., 2014; Ono et al., 2019), but few studies have examined its applicability to assess metal mixture toxicity (Koppel et al., 2019). Applied to REE mixtures, the CA or IA models, which no longer hold when the dose is expressed as the fraction of BLs occupied by the REE ions, may be valid when using the labile metal level as the dose expression. To our knowledge, dynamic aspects have rarely been considered in constructing models for predicting the mixture effects of REEs in soil.

Among the most abundant REEs, cerium (Ce) and yttrium (Y) represented light and heavy REEs, respectively, were selected in the current study. Our objective is to examine the toxicity of Y, Ce, and their binary mixtures to wheat (*Triticum aestivum* L.) and to develop a generic model that is capable of predicting their toxicity in different soils. To do so, the performance of conceptual models (CA and IA) in predicting and reconciling the Y–Ce mixture interplays and toxicity across different soil types were evaluated. For better understanding the bioavailability of REEs in a range of soils of varying properties, toxicity predictions were generated using different dose descriptors: three equilibrium-based (total metal concentrations in soil $[M]_{tot}$, free metal ion activities in soil solution $\{M^{3+}\}$ and the fraction (f) of metal ions bound to the BLs) and one kinetically controlled (DGT-induced REEs flux $[M]_{flux}$) dose. Assuming that the assumption of equilibrium under the BLM framework is invalid in complex soils, the kinetically labile metal pools are expected to represent the actual bioavailability of REEs in different soils.

2. Materials and methods

2.1. Experimental design

The plant root elongation tests (5 days) were performed to assess Y and Ce mixture toxicity and interactions in four selected soils with varying properties (Table S1). Specifically, they cover a wide range of pH_{CaCl2} (4.40–7.22), organic matter (OM) content (3.63–12.11%) and cation exchange capacity (CEC) (8.62–30.12 $cmol_c/kg$) that are expected to affect metal mixture interactions. A partial factorial design together with a ray design was set up to assess mixture toxicity and interactions in binary Y–Ce mixtures (see Table S2 for details).

2.2. Soil preparation

Three natural soil samples were collected from Nanjing, Dongbei, and Shanxi in China. In addition, an OECD standardized soil was prepared by mixing sphagnum peat (10%), kaoline clay (20%), and quartz sand (70%) (ISO, 1994). The pH of the OECD soil were adjusted to approximately 7.0 by the addition of $CaCO_3$. All soil samples were air dried, sieved at 2 mm and mixed to ensure homogeneity. The four different soils were spiked with appropriate amounts of rare-earth chloride ($YCl_3{\cdot}6H_2O$ or $CeCl_3{\cdot}7H_2O$; purity $>$ 99.99%) to obtain stock soils (10 kg soil for each REEs). The spiked soils were then incubated for 4 weeks at 20 °C, followed by leaching with artificial rainwater to remove excess salinity (Oorts et al., 2007). The non-spiked soils were mixed with different quantities of stock soils to obtain soil treatments with the target concentrations of REEs (Table S2). For the individual treatments in 4 soils, nominal concentrations ranged from 300 to 4500 mg/kg for Y and from 500 to 4000 mg/kg for Ce. For the Y–Ce mixture, nominal concentrations ranged from 150 to 1200 mg/kg for Y and from 250 to 1500 mg/kg for Ce in Nanjing soil, from 200 to 2500 mg/kg for Y and from 200 to 3200 mg/kg for Ce in Dongbei soil, from 200 to 1800 mg/kg for Y and from 200 to 1800 mg/kg for Ce in OECD soil, and from 300 to 2400 mg/kg for Y and from 200 to

2400 mg/kg for Ce in Shanxi soil. All the soil treatments were mixed homogeneously and incubated for another 4 weeks. After incubation, the soil was transferred to the test pots (3 replicates for each treatment) while maintaining moist conditions for each pot.

2.3. Toxicity bioassays

Wheat (*Triticum aestivum* L.) is one of the most important cereal crops in the world. It has been widely used as the model plant for toxicity tests (Gong et al. 2018, 2019; Wang et al., 2012; Wu et al., 2017). The wheat seeds were germinated for 48 h at 25 °C in darkness on paper moistened with deionized water. After 48 h, three seedlings with initial root length of ~1.2 cm were sown in three replicated pots for each soil treatment. Soils were initially moistened to reach 60% of the maximum water holding capacity. All the pots were placed randomly in a climate chamber at 20 °C with 75% relative humidity and an 8 h-light: 16 h-dark cycle. The plants grew for 5 days in the pots and deionized water was added daily to compensate for evapotranspiration. After exposure, the relative root elongation (RE, % of control) was calculated to determine the toxicity of Y, Ce and mixtures to wheat:

$$RE(\%) = \frac{RL_M}{RL_C} \times 100 \quad (1)$$

where RL_M (cm) is the average root length of the three seedlings exposed to REEs, and RL_C (cm) is the mean root length in the controls.

2.4. Diffusive gradients in thin films

DGT devices were purchased from DGT Research Ltd (Lancaster, UK). The assembled probes had an exposure window of 2.54 cm^2, a diffusive layer thickness of 0.078 cm and a poly (ether sulfone) filter membrane thickness of 0.014 cm. Each DGT device was deployed in 50 g of each treated soil that was wetted to 100% maximum water holding capacity with deionized water. The filter membrane of the DGT device was pushed into the soil to a depth of 1 cm and kept at 20 °C for 24 h. After 24 h, the DGT probes were rinsed with deionized water to remove soil particles, and then the resin gels were retrieved and immersed in 1 mL of 1 M HNO_3 for 24 h before analysis (Zhang et al., 1998). After deployments, soil porewater was collected by centrifugation (15 min at 3000 g) and then filtered through a 0.45 μM membrane filter.

The time-averaged concentration, that is, the DGT-induced metal flux $[M]_{flux}$ (μg/m^2/h) in soil porewater at the surface of the device was calculated:

$$[M]_{flux} = \frac{M \times 3600 \times 10\ \wedge\ 4}{A \times t} \quad (2)$$

where *M* represents the accumulated mass (μg) of Y/Ce in the resin gel, *A* represents exposure window (2.54 cm^2) and *t* represents the deployment time (s).

2.5. Chemical analysis and speciation calculations

For the leached soils, total Y/Ce concentrations in the DGT eluates, soil solutions and soil samples after digestion with *aqua regia* were determined with ICP-OES (iCAP7600, Thermo Fisher) and, if needed, ICP-MS (iCAP Q, Thermo Fisher). For quality control, a multi-element calibration standard (J&K Scientific Ltd.) and a reagent blank were analyzed every 25 samples. Soil pH in 0.01 M $CaCl_2$ extracts and in porewater samples was measured using a pH meter (Mettler Delta 320, Switzerland) at the end of the test. Some parallel soil samples were taken before, during, and after the tests. No noteworthy differences in soil pH were observed among different sampling periods. Before the experiment, soil texture, OM content, and CEC were determined for the non-spiked soils (see notes to Table S1 for details). Dissolved organic matter in soil porewater was determined by a TOC analyzer (multi 3100, Analytik Jena). Concentrations of dissolved Ca, Na, Mg, and K in soil porewater were measured by ICP-OES (Table S2). The Windermere Humic Aqueous Model VII (CEH, UK) was used to calculate the activities of Y^{3+}, Ce^{3+}, and other cations in soil porewater (see Notes to Table S2 for details) (Tipping et al., 2011).

2.6. Data analysis and model development

Dose-response curves for single Y and Ce exposures were fitted using a logistic model (Haanstra et al., 1985):

$$RE\ (\%) = \frac{100}{1 + \left(\frac{c}{EC50}\right)^{\beta}} \quad (3)$$

where *c* represents different dose expressions, such as total metal concentrations in soil $[M]_{tot}$ (mg/kg) or free metal ion activities in soil solution $\{M^{3+}\}$ (μmol/L), β is the slope of the derivative of the dose-response curve at c = EC50, and EC50 is the corresponding dose yielding a 50% inhibitory effect (mg/kg or μmol/L). First, eq (3) was fitted to the single Y or Ce toxicity data for each of the four different soils. Next, eq (3) was further fitted to the single REE toxicity data for all soils together. The derived EC50 and β values for Y or Ce in single exposure across all soils were then used to predict Y–Ce mixture toxicity in different soils with the reference models of CA and IA.

2.6.1. CA and IA

The CA concept was used to describe REE mixture toxicity assuming the components have the same or a similar mode of toxic action (i.e., act on the same biological pathway and strictly the same molecular target):

$$TU_{mix} = \sum_{i=0}^{n} \frac{c_i}{ECx_i} = \frac{c_Y}{EC50_Y} + \frac{c_{Ce}}{EC50_{Ce}} \quad (4)$$

where *n* is the number of mixture components, c_i is the concentration of component i in the mixtures causing x_i% effect, ECx_i is the effective concentration of component i causing x_i% effect when applied singly. The term $\frac{c_i}{ECx_i}$ is also defined as Toxic Unit (TU) which scales the relative toxicity of each component. TU_{mix} is therefore a dimensionless quantity that equals the sum of the TUs of the different components in the mixture. When the EC50 of the mixture equals 1 TU_{mix}, no antagonistic or synergistic effect occurs and CA holds. Eq (3) can be rewritten as follows:

$$\frac{c_Y}{EC50_Y \times \left(\frac{100 - RE_{mix}}{RE_{mix}}\right)^{1/\beta_Y}} + \frac{c_{Ce}}{EC50_{Ce} \times \left(\frac{100 - RE_{mix}}{RE_{mix}}\right)^{1/\beta_{Ce}}} = 1 \quad (5)$$

where RE_{mix} represents the predicted mixture response.

The concept of IA was applied to predict mixtures where the components have different modes of action (i.e., act on different physiological systems):

$$E(c_{mix}) = 1 - \prod_{i=1}^{n} [1 - E(c_i)] \quad (6)$$

where E (c_{mix}) is the predicted effect (scaled in the range of 0–1) of the mixture, c_i is the concentration of component i, E (c_i) is the effect of component i present singly at a concentration c_i. Eq (6) can be rewritten as follows when RE (%) was used as the toxicity endpoint:

$$REmix\ (\%) = \prod_{i=1}^{n} (RE_i) = \frac{RE_Y \times RE_{Ce}}{100} \quad (7)$$

where RE_Y and RE_{Ce} represent the predicted effects using eq (3) for Y or Ce applied singly.

2.6.2. BLM-based CA and IA approach

The principal feature in the BLM is the competition of the free metal ion with other cations for binding at the biotic ligand. In a BLM, single

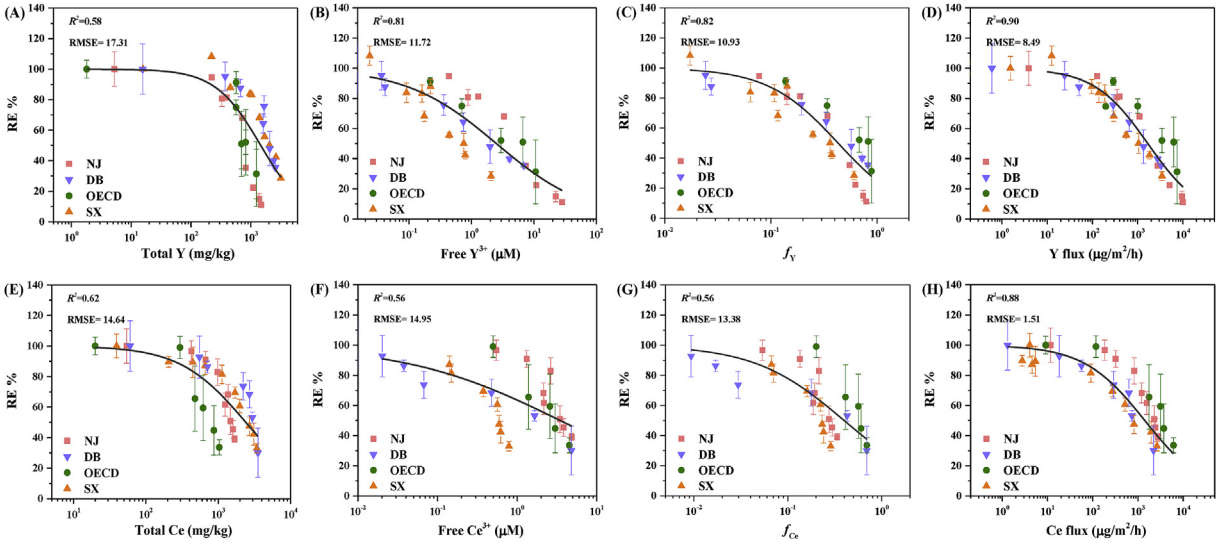

Fig. 1. Dose-response curves for the toxicity of Y or Ce to wheat (*Triticum aestivum*) root elongation in four different soils (see Table S1) after 5-d exposure based on different dose descriptors: total soil metal concentrations (A, E), free ion activities in soil porewater (B, F), fractions of metal occupied biotic ligands (C, G) and DGT-induced metal flux (D, H). The solid lines are logistic regression fits based on all soil toxicity data from all test soils together.

metal toxicity can be expressed as a function of the fraction (f) of the total BLs occupied by the toxic metal (Niyogi and Wood, 2004):

$$f_{MBL} = \frac{K_{MBL}\times\{M^{3+}\}}{1 + K_{MBL}\times\{M^{3+}\} + \sum K_{XBL}\times\{X^{Z+}\}} \quad (8)$$

where K_{MBL} is the stability constant for binding of the REE ion (M^{3+}) to the BL, K_{XBL} is the stability constant for a competing cation (X^{Z+}) for binding to the BL, and the curly brackets {} represent the corresponding free ion activities in soil porewater (mol/L).

The single-metal BLM can be further modified and extended to the BLM-based CA approach for quantifying metal mixture toxicity by considering both the competition between toxic metals and the competition between cations and toxic metals as follows:

$$f_{mix} = \frac{K_{YBL}\times\{Y^{3+}\} + K_{CeBL}\times\{Ce^{3+}\}}{1 + K_{YBL}\times\{Y^{3+}\} + K_{CeBL}\times\{Ce^{3+}\} + \sum K_{XBL}\times\{X^{Z+}\}} \quad (9)$$

where f_{mix} is the fraction of BLs occupied by Y^{3+} and Ce^{3+}; K_{YBL}, K_{CeBL}, and K_{XBL} are the stability binding constants of Y^{3+}, Ce^{3+} and X^{Z+} to the BLs (L/mol), respectively, and curly brackets {} are the corresponding free ion activities in soil porewater (mol/L). Substituting this expression into eq (3) yields

$$REmix\ (\%) = \frac{100}{1 + \left(\frac{f_{mix}}{f_{mix50}}\right)^{\beta_{mix}}} \quad (10)$$

According to the BLM-based IA approach, in a first step eq (3) was used to predict the response of single metal. This yields:

$$RE\ (\%) = \frac{100}{1 + \left(\frac{f_{MBL}}{f_{MBL}^{50\%}}\right)^{\beta_i}} \quad (11)$$

In a second step, REE mixture toxicity was predicted in the IA framework by substituting eq (11) into eq (7). This yields:

$$REmix\ (\%) = \frac{100}{1 + \left(\frac{f_{YBL}}{f_{YBL}^{50\%}}\right)^{\beta_Y}} \times \frac{1}{1 + \left(\frac{f_{CeBL}}{f_{CeBL}^{50\%}}\right)^{\beta_{Ce}}} \quad (12)$$

The estimations of the BLM parameters ($\log K_{XBL}$, $\log K_{MBL}$, β_i, $f_{MBL}^{50\%}$ and f_{mix50}) were based on multiple non-linear regression. The regression modeling followed the procedures of JMP 16.0 (SAS Institute, US) by solving the values of these parameters to minimize the root-mean-square error (RMSE). Among the co-existing protons and cations (i.e., H^+, Ca^{2+}, Mg^{2+}, Na^+, and K^+), H^+ was identified as potential competing proton and included into the BLM in a stepwise manner in which the model performance was significantly improved.

3. Results and discussion

3.1. Soil properties and control performance

Soil properties such as pH, cation exchange capacity (CEC), organic matter (OM) content and soil texture, are crucial factors that drive metal bioavailability, and subsequently influence interactions between metal mixture components in soils (Peijnenburg et al., 2012). Therefore, four soils with contrasting properties were selected to identify the diverse interactive effects of Y–Ce mixtures at the exposure level. The selected properties of the non-spiked soils are presented in Table S1. The total Y and Ce concentrations in the four soil samples varied from 1.8 to 15 and from 19 to 60 mg/kg, respectively. Of the four soils used for all experiments, two acidic soils including Nanjing (NJ) and Dongbei (DB) soil with pH_{CaCl2} of 4.40 and 5.98, respectively, one nearly neutral soil (OECD soil) with pH of 6.75, and one alkaline soil of Shanxi (SX) with pH of 7.22. The measured values of the CEC and OM content of NJ, DB, OECD and SX soil were 20.01, 30.12, 8.62, 16.34 $cmol_c$/kg and 11.34, 12.11, 3.63, 6.00%, respectively (Table S1). The root lengths (normally 11–12 cm) of wheat grown in the non-spiked OECD soil were similar to those grown in other non-spiked natural soils. This suggests that the natural soils were not contaminated.

3.2. Bioavailability and toxicity of individual Y and Ce

In the present study, four different expressions of exposure ($[M]_{tot}$, $\{M^{3+}\}$, f, and $[M]_{flux}$) were considered to evaluate bioavailability and toxicity of individual Y and Ce based on all soil toxicity data (Fig. 1). Reasonable fits ($R^2 > 0.84$) were obtained by using the logistic function to describe the toxicity data of the single REEs in each soil (Table 1). When further applying this model to fit single Y toxicity data in all soils together using $\{M^{3+}\}$ as the dose, the model provided superior fits ($R^2 = 0.81$ and RMSE = 11.72) in comparison to the fits obtained using $[M]_{tot}$ as the dose ($R^2 = 0.58$ and RMSE = 14.64). However, for single Ce data in all soils, the model fit became slightly weaker when using $\{M^{3+}\}$ as the dose ($R^2 = 0.56$ and RMSE = 14.95) as compared to the $[M]_{tot}$ ($R^2 = 0.62$ and RMSE = 14.64). At least, our results showed that the total soil concentration failed to explain well

Table 1
Dose-response parameter values (EC50 and β) and model fits (R^2 and RMSE) for the toxicity of Y and Ce to wheat (*Triticum aestivum*) root elongation in different soils, expressed on the basis of total metal concentrations (mg/kg), free ion activities (μmol/L) and DGT-induced metal flux (μg/m^2/h), respectively.[a]

		NJ	DB	OECD	SX	all soils
total Y	EC50	735 (48.4)[d]	2094 (85.8)	860 (75.7)	2071 (94.8)	1461 (212)
	β	2.60 (0.42)	2.77 (0.56)	2.82 (0.85)	1.98 (0.22)	1.14 (0.27)
	R^2[b]	0.95	0.94	0.84	0.96	0.59
	RMSE[c]	6.94	5.09	8.54	4.37	17.31
total Ce	EC50	1522 (38.1)	3029 (135)	752 (48.9)	2531 (128)	2411 (400)
	β	3.38 (0.47)	4.22 (1.21)	2.24 (0.43)	1.61 (0.18)	0.96 (0.20)
	R^2	0.96	0.89	0.94	0.96	0.62
	RMSE	3.87	5.38	3.98	6.76	14.64
free Y^{3+}	EC50	4.69 (0.39)	2.08 (0.18)	4.33 (0.77)	0.68 (0.10)	2.51 (0.49)
	β	1.17 (0.10)	0.59 (0.04)	0.66 (0.10)	0.99 (0.17)	0.60 (0.08)
	R^2	0.98	0.99	0.96	0.91	0.81
	RMSE	3.94	4.39	7.04	2.26	11.72
free Ce^{3+}	EC50	3.73 (0.35)	1.48 (0.39)	2.80 (0.26)	0.57 (0.04)	3.50 (1.44)
	β	1.83 (0.43)	0.50 (0.07)	1.43 (0.28)	1.44 (0.25)	0.45 (0.13)
	R^2	0.87	0.95	0.95	0.93	0.56
	RMSE	7.38	5.06	5.23	4.79	14.95
Y flux	EC50	1724 (109)	1408 (78.5)	4074 (1160)	1027 (126)	1685 (195)
	β	1.08 (0.06)	0.68 (0.03)	0.63 (0.15)	0.80 (0.09)	0.73 (0.07)
	R^2	0.99	0.99	0.87	0.96	0.90
	RMSE	2.75	7.27	4.63	1.68	8.49
Ce flux	EC50	2180 (61.6)	1007 (157)	3484 (261)	948 (170)	1423 (165)
	β	1.44 (0.09)	0.81 (0.14)	1.14 (0.19)	0.53 (0.07)	0.64 (0.07)
	R^2	0.99	0.95	0.98	0.95	0.88
	RMSE	1.86	3.27	4.62	4.60	1.51

[a] See Table S1 for soil properties.
[b] R^2 is the coefficient of determination for the linear regression between the predicted and observed RE (%).
[c] RMSE indicates the root-mean-square error of the predicted RE (%).
[d] Standard errors are indicated in brackets.

the variation in single Y or Ce toxicity across soils ($R^2 \sim 0.60$). There is extensive evidence showing that the total concentration of a metal in soils is a poor indicator of its toxicity, resulting in either underestimation or overestimation of the actual risk (Gopalapillai and Hale, 2017; Qiu and He, 2017; Qiu et al., 2016). Although free ion activities are often considered to be the immediately available metal fractions and can represent the bioavailability much better, free ion activities failed to explain a relatively large proportion of variation in the observed Ce toxicity in all soils together. Other toxicity-modifying factors such as co-existing cations and pH might have affected Ce bioavailability and thus its toxicity when all single Ce data in soils were considered.

The BLM concept was therefore applied as an alternative to estimate the single toxicity of Y/Ce in different soils considering not only chemical speciation but also ion competition. The values of $\log K_{HBL}$, $\log K_{YBL}$, and $\log K_{CeBL}$ were estimated to be 4.96, 5.84, and 5.71, respectively (Table 2). The BLM provided a feasible fit for modeling Y toxicity to wheat in different soils, with $R^2 = 0.82$ and RMSE = 10.93. However, after considering the competitive effect of H^+ for single Ce toxicity in all soils, the model fit was still poor with $R^2 = 0.56$ and RMSE = 13.44. Our study showed intriguing results that the BLM could reasonably predict Y toxicity but not Ce toxicity across different soils. Metal speciation changes substantially over the alkaline pH range and species other than the metal ion may dominate in alkaline solutions (Wang et al., 2012). In addition to $\{Ce^{3+}\}$, other metal forms may also contribute to toxicity at pH-values exceeding 7, leading to underestimation of metal toxicity in alkaline soil solutions based on $\{M^{3+}\}$. Indeed, it was found that above pH 6.5 it was not possible to explain Sc bioavailability to *Chlamydomonas reinhardtii* without considering the contribution of hydroxo complexes (i.e., $Sc(OH)^{2+}$, $Sc(OH)_2^+$, $Sc(OH)_3$) (Cremazy et al., 2013). Therefore, other toxic species of Ce that can be resupplied from the soil solid phase into the solution should be taken into account.

The kinetically controlled dose $[M]_{flux}$ was applied to model single Y/Ce toxicity data in each of the four soils and in all soils together.

Table 2
Summary of model fits associated with the biotic ligand model (BLM) calibrated to single Y and Ce toxicities to wheat (*Triticum aestivum*) root elongation in all four test soils together.

model	Binding constant (log *K*) values			$f_{mix}^{50\%}$[b]		β[c]		R^2[d]	RMSE[e]
	H-BL[a]	Y-BL	Ce-BL						
BLM-CA	4.96	5.84	5.71	0.31		1.21		0.68	10.75
				$f_Y^{50\%}$	$f_{Ce}^{50\%}$	β_Y	β_{Ce}		
BLM-IA				0.41	0.38	1.27	0.91	0.69	13.12

[a] BL represents biotic ligand.
[b] $f_{MBL}^{50\%}$ indicates the fraction of biotic ligands occupied by Y^{3+} and Ce^{3+} singly or in binary mixture that cause 50% growth reduction.
[c] β_M represents the model slope.
[d] R^2 is the coefficient of determination for the linear regression between the predicted and observed RE (%).
[e] RMSE indicates the root-mean-square error of the predicted RE (%).

Considerable improvements of the model fits were observed when modeling single Y/Ce toxicity data in all soils together based on $[M]_{flux}$. The resulting values of R^2 and RMSE were, respectively, 0.90 and 8.49 for Y, and 0.88 and 1.51 for Ce. Due to the complex impacts on metal partitioning and speciation resulting from the soil properties, accurate evaluation of metal bioavailability in soil is challenging (Oorts et al., 2006; Peijnenburg et al.; Smolders et al., 2009). A variety of chemical extraction methods are available for estimating bioavailable fractions of metals in soil (Peijnenburg and Vijver, 2007). However, the information on labile metal provided by these methods varies with the metal, the chemical species, and the soil contamination extent (Wang et al., 2002). Further to this, chemical extraction methods focus on the static equilibrium of metals between the solid phase and the solution phase. However, under natural conditions, the uptake of metals by plants is a complicated process, which is mainly controlled by diffusion (Davison and Zhang, 2012). If metal diffusion through the diffusive boundary

layer nearby the roots becomes the rate-limiting step for uptake, the free ion activity at the microenvironment interface will differ from that in the bulk solution (Degryse et al., 2012). As a result, the diffusion limitation might disturb the dynamic equilibrium and promote release of metals from the soil solid phase to the solution phase. Therefore, it is necessary to consider the kinetically labile metal when modeling metal toxicity across different soils. The DGT technique is able to mimic the process of metal uptake by plants in soil, which provides a measurement for the diffusive supply of metals in the context of an infinite sink (i.e. the binding gel in the DGT device), thus promoting the release of labile metals from the solid phase of the soil (Degryse et al., 2009). The results of the present study indeed demonstrated that only use $\{Ce^{3+}\}$ cannot represent actual Ce bioavailable species in different soils, but kinetics issues were able to solve the 'problem' encountered for modeling Ce toxicity described above. This suggests that the kinetically controlled dose was a reliable indicator of bioavailability of individual REEs in different soils.

3.3. Y–Ce mixture interactions and toxicity across soils

The MOAs of Y and Ce in soil are incompletely known. Therefore, both CA and IA models were considered to predict the mixture toxicity of Y and Ce to wheat in the four soils separately (Fig. 2) and in all soils together (Fig. 3) based on the estimated parameter values (EC50 and β) from the individual REE exposures (Table 1). The conceptual models of CA and IA associated with different expressions of exposure ($[M]_{tot}$, $\{M^{3+}\}$ and $[M]_{flux}$) were compared. In general, the occurrence of interplays can be identified when the observed mixture effects deviate from the predictions using conceptual models (Qiu and He, 2017).

The accuracies of CA and IA in modeling mixture toxicity were similar (Table S3). The possible explanation is that only the binary mixtures were modelled. If the CA and IA model are applied to predict the toxicity of higher-order mixtures (e.g., ternary, quaternary combinations), larger differences would be expected according to the mathematical expressions of the two reference models (Backhaus and Faust, 2012; Silva et al., 2002). Therefore, we propose that one should pay more attention to the mathematical connotations instead of the mechanistic meanings behind the CA and IA models. In each soil, the two reference models did not always point at the same patterns of deviation from the additivity. The deviation patterns of Y and Ce depended on the type of soils and expressions of exposure applied. With $[M]_{tot}$ as the dose metric, nearly additive effects were observed after CA modeling except for the OECD soil, which was the only soil that showed slightly less-than-additive effects at lower dose, changing to synergistic responses at higher dose. Meanwhile, the IA-predicted responses were larger than the observed responses, suggesting more-than-additive effects in all the soils except the SX soil, which was the only soil that showed nearly additive effects. In the case of a low metal content, soil sorption might immobilize the metals, thereby alleviating their toxicity, leading to the less-than-additive effect observed in OECD soil at lower dose on the basis of $[M]_{tot}$. The toxicity of a metal could be aggravated by the addition of a second metal by increasing its bioavailability, leading to more-than-additive effects (Qiu et al. 2011, 2016). With $\{M^{3+}\}$ as the dose metric, the patterns of deviation from the additivity were almost similar for CA and IA, indicating nearly additive effects in all soils except for SX soil where synergistic responses were observed. Predicted results that displayed deviations from additivity were obtained in different soils using $[M]_{tot}$ and $\{M^{3+}\}$ as the dose expression. This suggests that interplays of Y–Ce mainly occurred at the exposure level in the soil matrix. Indeed, the finding that the DGT-induced flux of one metal was increased by the presence of the other metal, indicated there were interactions of Y and Ce at the exposure level (Fig. S1). Similar conclusions have for instance been reported for Cd water extractability considerably increasing by the addition of Zn to soil (Van Gestel and Hensbergen, 1997). By separating the interactions at the exposure level from interactions at the uptake and target levels, it was possible to determine where the interactions possibly occur. This allowed to subsequently assess their influence on the toxicity pattern of binary metal mixtures. These findings will be beneficial to reconcile the interplays and toxicity of metal mixtures among different soils.

In all soils together, using $[M]_{tot}$ as the dose expression representing the bioavailable fraction, poor fits were observed with R^2 of 0.41 and RMSE of 11.68 for CA, and R^2 of 0.41 and RMSE of 13.13 for IA (Fig. 3A, E; Table S3). When using $\{M^{3+}\}$ as dose, the goodness of fit was improved with the value of R^2 increasing, respectively, from 0.41 to 0.64 for CA, and from 0.41 to 0.63 for IA (Fig. 3B, F; Table S3). Possible interplays between Y and Ce limited the predictive capability of the simple conceptual models coupled with two equilibrium-based doses among different soils. Therefore, attempts were made in our study to develop a bioavailability-based BLM model incorporating mixture interactions for predicting toxicity of Y–Ce mixtures. The BLM theory has rarely been applied to metal mixture toxicity data generated in soil (Qiu et al. 2015, 2016). It is challenging to univariately modify the parameters that influence metal toxicity in soil systems as there is covariance among most of the physicochemical properties of soils (Lofts et al., 2004). In our study, the optimal values of the BLM were obtained as follows: $f_{YBL}^{50\%} = 0.41$, $f_{CeBL}^{50\%} = 0.38$, $f_{mix}^{50\%} = 0.31$, $\beta_Y = 1.27$, $\beta_{Ce} = 0.91$, and $\beta = 1.21$ (Table 2). These models gave reasonable predictions with R^2 of 0.68 and RMSE of 10.75 for BLM-based CA, and R^2 of 0.69 and RMSE of 13.12 for BLM-based IA (Fig. 3C, G; Table 2). About 30% of the variance in toxicity was thus left unexplained in our study, challenging the view that the free metal ion is the main bioavailable form. The possible reason might be lie in the fact that the BLM was initially developed for aqueous environments with the assumption of equilibrium partitioning. This assumption might not be valid when the environmental bioavailability can be affected by the dissolution kinetics of a metal from the soil solid phase towards the soil solution phase. Therefore, the basic assumptions of the BLM may be not applicable for soils.

Based on the above considerations, CA and IA in combination with the kinetically controlled dose ($[M]_{flux}$) were used to model the whole set of mixture data generated in the four soils. This provided the best model fits with R^2 of 0.80 and RMSE of 11.48 for CA, and R^2 of 0.78 and RMSE of 13.89 for IA (Fig. 3D, H; Table S3). In the DGT device, the binding gel was generally regarded as an infinite sink with no competition at the gel, however, competition effects were indeed observed in some situations (Jimenez-Piedrahita et al., 2017). In our study, a dynamic competition between Y^{3+} and Ce^{3+} for the binding sites at the surface of the binding layer might exist as they possessed similar selectivity for the Chelex resin (Oyvind Aaberg et al., 2003). Similarly, competitive effects of Fe^{2+} and Mn^{2+}/Cd^{2+} on the DGT binding resin were observed in a previous study (Koppel et al., 2019). The competition effects can be influenced by the relative metal concentrations and the selectivity of a metal for the Chelex resin. The flux of metal from soil during the deployment period obtained by the DGT measurement can provide adequate information on the dynamic bioavailability of Y and Ce in soils of varying properties, reflecting the concentration in the soil porewater and the resupply of labile species from the solid phase to solution (Simpson et al., 2012). The variations in toxicity due to changes in the size of the labile metal pool substantially attenuated based on $[M]_{flux}$ in different soils ($R^2 \sim 0.80$), indicating that the dynamic bioavailability concept could robustly explain the Y–Ce mixture interplays and toxicity to wheat among the different soils used in our study.

4. Conclusions

In the present study, bioavailability models were constructed for predicting Y–Ce mixture toxicity across soils. When the whole set of mixture toxicity data were fitted, poor model fits were obtained using CA and IA ($R^2 < 0.64$) based on either $[M]_{tot}$ or $\{M^{3+}\}$, while the use of a mechanistic framework (the BLM-based CA and IA approach) gave

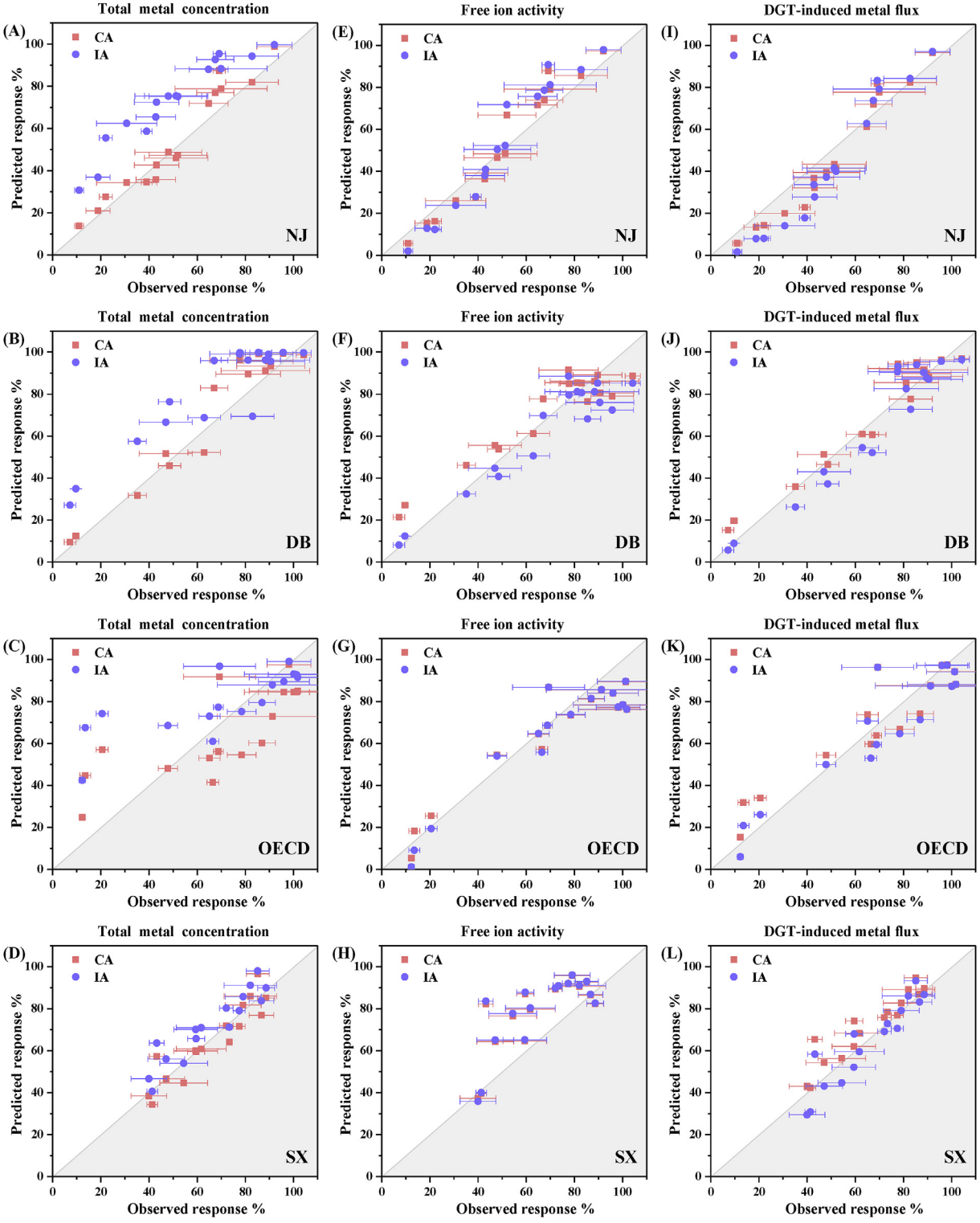

Fig. 2. Prediction of the toxicity of Y and Ce to wheat (*Triticum aestivum*) root elongation using the concentration addition (CA) and independent action (IA) reference models based on total metal concentrations (A, B, C, D), free ion activities (E, F, G, H), and DGT-induced metal flux (I, J, K, L) for the four test soils separately.

reasonable predictions ($R^2 \sim 0.70$). The unexplained variance in toxicity indicated that the equilibrium assumption under the BLM framework may not be justified in complex soils. When the kinetically controlled dose metric ($[M]_{flux}$) integrating free metal ions and labile inorganic/organic complexes was adopted, the remaining variance in toxicity was substantially attenuated in all soils ($R^2 \sim 0.80$). Our study suggests that the basic assumption underlying the BLM may need reconsideration when predicting Y–Ce mixture toxicity in different soils. In soil-plant systems, a dynamic bioavailability analysis could provide a robust basis for modeling and reconciling the interplays and toxicity of mixtures of Y and Ce. In addition, given the use of one standardized OECD soil, it would be possible to extrapolate our conclusions to different soils. In our study, the dynamic bioavailability still could not fully explain the overall variations in Y–Ce mixture toxicity across soils, which might be attributed to interactions at the uptake level and the target level. Therefore, further research on the toxicokinetic and toxicodynamic

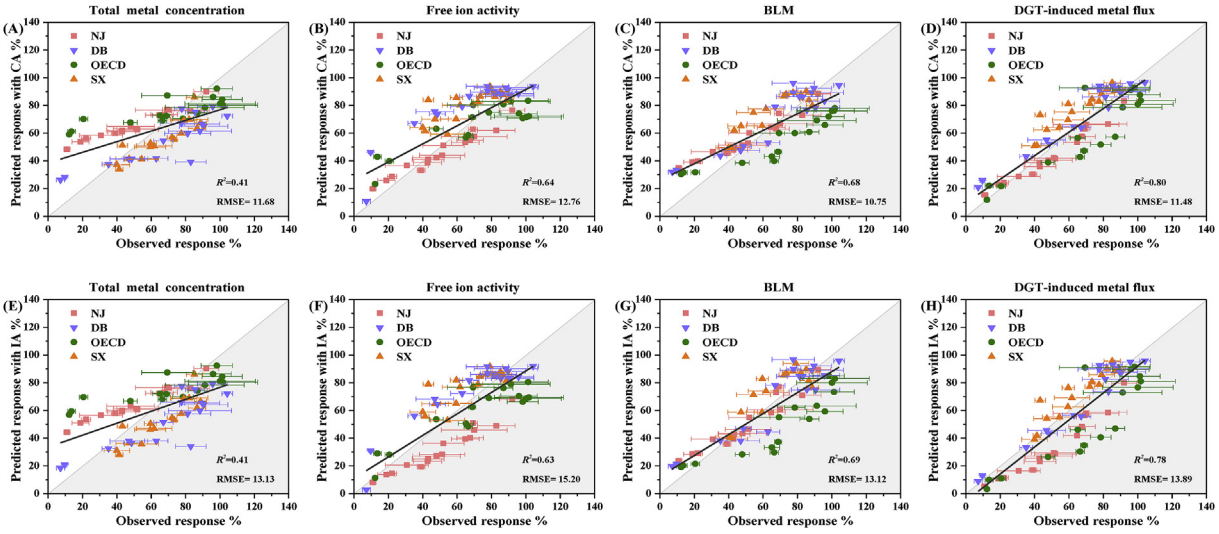

Fig. 3. Prediction of the toxicity of mixtures of Y and Ce to wheat (*Triticum aestivum*) root elongation using the frameworks of concentration addition (CA) and independent action (IA) based on total metal concentrations (A, E), free ion activities (B, F), fractions of metal occupied biotic ligands (C, G) and DGT-induced metal flux (D, H) for all the four soils together.

processes of REEs is required. Our work provides valuable soil toxicity datasets of REEs, enriching the scientific knowledge on REEs bioavailability and mixture toxicity in soils.

Author contribution statement

Bing Gong: Data curation; Formal analysis; Writing - original draft; Erkai He: Methodology; Writing - review & editing; Willie J.G.M. Peijnenburg: Writing - review & editing; Yuichi Iwasaki: Writing - review & editing; Cornelis A.M. Van Gestel: Writing - review & editing; Xinde Cao: Project administration; Ling Zhao: Co-supervision; Xiaoyun Xu: Co-supervision; Hao Qiu: Conceptualization; Supervision; Writing - review & editing; Funding acquisition

Acknowledgements

This study was supported by the National Natural Science Foundation of China (No. 41701573, No. 41701571, No. 41877500, and No. 41977115), the National Key R&D Program of China (No. 2018YFC1800600, No. 2018YFD0800700), the Research Fund Program of Guangdong Provincial Key Laboratory of Environmental Pollution Control and Remediation Technology (No. 2018K01), Science and Technology Program of Guangzhou, China (No. 201904010116), and Fundamental Research Funds for the Central Universities (No. 19lgpyl50).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2020.109736.

References

Adeel, M., Lee, J.Y., Zain, M., Rizwan, M., Nawab, A., Ahmad, M.A., Shafiq, M., Yi, H., Jilani, G., Javed, R., Horton, R., Rui, Y., Tsang, D.C.W., Xing, B., 2019. Cryptic footprints of rare earth elements on natural resources and living organisms. Environ. Int. 127, 785–800.

Amundson, R., Berhe, A.A., Hopmans, J.W., Olson, C., Sztein, A.E., Sparks, D.L., 2015. Soil and human security in the 21st century. Science 348, 1261071.

Backhaus, T., Faust, M., 2012. Predictive environmental risk assessment of chemical mixtures: a conceptual framework. Environ. Sci. Technol. 46, 2564–2573.

Campbell, P.G., 1995. Interactions between trace metals and aquatic organisms: a critique of the free-ion activity model. In: Tessier, A., Turner, D. (Eds.), Metal Speciation and Bioavailability in Aquatic Systems. John Wiley, New York, NY, USA, pp. 45–102.

Cremazy, A., Campbell, P.G., Fortin, C., 2013. The biotic ligand model can successfully predict the uptake of a trivalent ion by a unicellular alga below pH 6.50 but not above: possible role of hydroxo-species. Environ. Sci. Technol. 47, 2408–2415.

Cremazy, A., Brix, K.V., Wood, C.M., 2019. Using the biotic ligand model framework to investigate binary metal interactions on the uptake of Ag, Cd, Cu, Ni, Pb and Zn in the freshwater snail *Lymnaea stagnalis*. Sci. Total Environ. 647, 1611–1625.

Davison, W., Zhang, H., 2012. Progress in understanding the use of diffusive gradients in thin films (DGT) - back to basics. Environ. Chem. 9, 1–13.

De Schamphelaere, K.A., Janssen, C.R., 2002. A biotic ligand model predicting acute copper toxicity for *Daphnia magna*: the effects of calcium, magnesium, sodium, potassium, and pH. Environ. Sci. Technol. 36, 48–54.

Degryse, F., Smolders, E., Zhang, H., Davison, W., 2009. Predicting availability of mineral elements to plants with the DGT technique: a review of experimental data and interpretation by modelling. Environ. Chem. 6, 198.

Degryse, F., Shahbazi, A., Verheyen, L., Smolders, E., 2012. Diffusion limitations in root uptake of cadmium and zinc, but not nickel, and resulting bias in the Michaelis constant. Plant Physiol. 160, 1097–1109.

Di Toro, D.M., Allen, H.E., Bergman, H.L., Meyer, J.S., Paquin, P.R., Santore, R.C., 2001. Biotic ligand model of the acute toxicity of metals. 1. Technical Basis. Environ. Toxicol. Chem. 20, 2383–2396.

Dinali, G.S., Root, R.A., Amistadi, M.K., Chorover, J., Lopes, G., Guilherme, L.R.G., 2019. Rare earth elements (REY) sorption on soils of contrasting mineralogy and texture. Environ. Int. 128, 279–291.

Drakvik, E., Altenburger, R., Aoki, Y., Backhaus, T., Bahadori, T., Barouki, R., Brack, W., Cronin, M.T.D., Demeneix, B., Hougaard Bennekou, S., van Klaveren, J., Kneuer, C., Kolossa-Gehring, M., Lebret, E., Posthuma, L., Reiber, L., Rider, C., Ruegg, J., Testa, G., van der Burg, B., van der Voet, H., Warhurst, A.M., van de Water, B., Yamazaki, K., Oberg, M., Bergman, A., 2020. Statement on advancing the assessment of chemical mixtures and their risks for human health and the environment. Environ. Int. 134, 105267.

Ernstberger, H., Zhang, H., Tye, A., Young, S., Davison, W., 2005. Desorption kinetics of Cd, Zn, and Ni measured in soils by DGT. Environ. Sci. Technol. 39, 1591–1597.

Gong, B., He, E., Qiu, H., Li, J., Ji, J., Zhao, L., Cao, X., 2018. Phytotoxicity of individual and binary mixtures of rare earth elements (Y, La, and Ce) in relation to bioavailability. Environ. Pollut. 246, 114–121.

Gong, B., He, E., Qiu, H., Li, J., Ji, J., Peijnenburg, W., Liu, Y., Zhao, L., Cao, X., 2019. The cation competition and electrostatic theory are equally valid in quantifying the toxicity of trivalent rare earth ions (Y^{3+} and Ce^{3+}) to *Triticum aestivum*. Environ. Pollut. 250, 456–463.

Gopalapillai, Y., Hale, B.A., 2017. Internal versus external dose for describing ternary metal mixture (Ni, Cu, Cd) chronic toxicity to *Lemna minor*. Environ. Sci. Technol. 51, 5233–5241.

Gui, X., Rui, M., Song, Y., Ma, Y., Rui, Y., Zhang, P., He, X., Li, Y., Zhang, Z., Liu, L., 2017. Phytotoxicity of CeO_2 nanoparticles on radish plant (*Raphanus sativus*). Environ. Sci. Pollut. Res. 24, 13775–13781.

Haanstra, L., Doelman, P., Voshaar, J.H.O., 1985. The use of sigmoidal dose response curves in soil ecotoxicological research. Plant Soil 84, 293–297.

Hatano, A., Shoji, R., 2008. Toxicity of copper and cadmium in combinations to Duckweed analyzed by the biotic ligand model. Environ. Toxicol. 23, 372–378.

ISO, 1994. Soil Quality - Effects of Soil Pollutants on Collembola (*Folsomia candida*): Method for the Determination of Effects on Reproduction. ISO/CD London, UK, pp. 11267.

Jho, E.H., An, J., Nam, K., 2011. Extended biotic ligand model for prediction of mixture toxicity of Cd and Pb using single metal toxicity data. Environ. Toxicol. Chem. 30, 1697–1703.

Jimenez-Piedrahita, M., Altier, A., Cecilia, J., Puy, J., Galceran, J., Rey-Castro, C., Zhang,

H., Davison, W., 2017. Extending the use of diffusive gradients in thin films (DGT) to solutions where competition, saturation, and kinetic effects are not negligible. Anal. Chem. 89, 6567–6574.
Komjarova, I., Blust, R., 2009. Multimetal interactions between Cd, Cu, Ni, Pb, and Zn uptake from water in the zebrafish *Danio rerio*. Environ. Sci. Technol. 43, 7225–7229.
Koppel, D.J., Adams, M.S., King, C.K., Jolley, D.F., 2019. Diffusive gradients in thin films can predict the toxicity of metal mixtures to two microalgae: validation for environmental monitoring in antarctic marine conditions. Environ. Toxicol. Chem. 38, 1323–1333.
Liang, T., Li, K., Wang, L., 2014. State of rare earth elements in different environmental components in mining areas of China. Environ. Monit. Assess. 186, 1499–1513.
Lofts, S., Spurgeon, D.J., Svendsen, C., Tipping, E., 2004. Deriving soil critical limits for Cu, Zn, Cd, and Pb: a method based on free ion concentrations. Environ. Sci. Technol. 38, 3623–3631.
Luo, J., Zhang, H., Zhao, F.-J., Davison, W., 2010. Distinguishing diffusional and plant control of cd and ni uptake by hyperaccumulator and nonhyperaccumulator plants. Environ. Sci. Technol. 44, 6636–6641.
Luo, J., Cheng, H., Ren, J., Davison, W., Zhang, H., 2014. Mechanistic insights from DGT and soil solution measurements on the uptake of Ni and Cd by radish. Environ. Sci. Technol. 48, 7305–7313.
Mihajlovic, J., Rinklebe, J., 2018. Rare earth elements in German soils - a review. Chemosphere 205, 514–523.
Morel, F., 1983. Principles of Aquatic Chemistry. John Wiley, New York, NY, USA.
Niyogi, S., Wood, C.M., 2004. Biotic ligand model, a flexible tool for developing site-specific water quality guidelines for metals. Environ. Sci. Technol. 38, 6177–6192.
Norwood, W.P., Borgmann, U., Dixon, D.G., Wallace, A., 2003. Effects of metal mixtures on aquatic biota: a review of observations and methods. Hum. Ecol. Risk Assess. 9, 795–811.
Ono, K., Yasutaka, T., Hayashi, T.I., Kamo, M., Iwasaki, Y., Nakamori, T., Fujii, Y., Kamitani, T., 2019. Model construction for estimating potential vulnerability of Japanese soils to cadmium pollution based on intact soil properties. PloS One 14, e0218377.
Oorts, K., Ghesquiere, U., Swinnen, K., Smolders, E., 2006. Soil properties affecting the toxicity of $CuC1_2$ and $NiCl_2$ for soil microbial processes in freshly spiked soils. Environ. Toxicol. Chem. 25, 836–844.
Oorts, K., Ghesquiere, U., Smolders, E., 2007. Leaching and aging decrease nickel toxicity to soil microbial processes in soils freshly spiked with nickel chloride. Environ. Toxicol. Chem. 26, 1130–1138.
Oyvind Aaberg, G., Oddvar, R.Y., Eiliv, S., Trond Peder, F., 2003. Performance study of diffusive gradients in thin films for 55 elements. Anal. Chem. 75, 3573–3580.
Peijnenburg, W.J.G.M., Vijver, M.G., 2007. Metal-specific interactions at the interface of chemistry and biology. Pure Appl. Chem. 79, 2351–2366.
Peijnenburg, W.J.G.M., Baerselman, R., de Groot, A.C., Jager, T., Posthuma, L., Van Veen, R.P.M., 1999. Relating environmental availability to bioavailability: soil-type-dependent metal accumulation in the Oligochaete *Eisenia andre*i. Ecotoxicol. Environ. Saf. 44, 294–310.
Peijnenburg, W., Capri, E., Kula, C., Liess, M., Luttik, R., Montforts, M., Nienstedt, K., Römbke, J., Sousa, J.P., Jensen, J., 2012. Evaluation of exposure metrics for effect assessment of soil invertebrates. Crit. Rev. Environ. Sci. Technol. 42, 1862–1893.
Playle, R.C., 2004. Using multiple metal-gill binding models and the toxic unit concept to help reconcile multiple-metal toxicity results. Aquat. Toxicol. 67, 359–370.
Qiu, H., He, E., 2017. Development of electrostatic-based bioavailability models for interpreting and predicting differential phytotoxicity and uptake of metal mixtures across different soils. Environ. Pollut. 226, 308–316.
Qiu, H., Vijver, M.G., Peijnenburg, W.J.M., 2011. Interactions of cadmium and zinc impact their toxicity to the earthworm *Aporrectodea caliginosa*. Environ. Toxicol. Chem. 30, 2084–2093.
Qiu, H., Vijver, M.G., He, E., Liu, Y., Wang, P., Xia, B., Smolders, E., Versieren, L., Peijnenburg, W.J.M., 2015. Incorporating bioavailability into toxicity assessment of Cu-Ni, Cu-Cd, and Ni-Cd mixtures with the extended biotic ligand model and the WHAM-F_{tox} approach. Environ. Sci. Pollut. Res. 22, 19213–19223.
Qiu, H., Versieren, L., Rangel, G.G., Smolders, E., 2016. Interactions and toxicity of Cu-Zn mixtures to *Hordeum vulgare* in different soils can be rationalized with bioavailability-based prediction models. Environ. Sci. Technol. 50, 1014–1022.
Romero-Freire, A., Minguez, L., Pelletier, M., Cayer, A., Caillet, C., Devin, S., Gross, E.M., Guérold, F., Pain-Devin, S., Vignati, D.A.L., Giamberini, L., 2018. Assessment of baseline ecotoxicity of sediments from a prospective mining area enriched in light rare earth elements. Sci. Total Environ. 612, 831–839.
Silva, E., Rajapakse, N., Kortenkamp, A., 2002. Something from "nothing" − eight weak estrogenic chemicals combined at concentrations below NOECs produce significant mixture effects. Environ. Sci. Technol. 36, 1751–1756.
Simpson, S.L., Yverneau, H., Cremazy, A., Jarolimek, C.V., Price, H.L., Jolley, D.F., 2012. DGT-induced copper flux predicts bioaccumulation and toxicity to bivalves in sediments with varying properties. Environ. Sci. Technol. 46, 9038–9046.
Smolders, E., Oorts, K., Van Sprang, P., Schoeters, I., Janssen, C., McGrath, S., McLaughlin, M., 2009. Toxicity of trace metals in soil as affected by soil type and aging after contamination: using calibrated bioavailability models to set ecological soil standards. Environ. Toxicol. Chem. 28, 1633–1642.
Sprecher, B., Daigo, I., Murakami, S., Kleijn, R., Vos, M., Kramer, G.J., 2015. Framework for resilience in material supply chains, with a case study from the 2010 rare earth crisis. Environ. Sci. Technol. 49, 6740–6750.
Thakali, S., Allen, H., M Di Toro, D., A Ponizovsky, A., P Rooney, C., Zhao, F.-J., McGrath, S., 2006. A terrestrial biotic ligand model. 1. Development and application to Cu and Ni toxicities to barley root elongation in soils. Environ. Sci. Technol. 40, 7085–7093.
Tipping, E., Lofts, S.E., Sonke, J., 2011. Humic Ion-Binding Model VII: a revised parameterisation of cation-binding by humic substances. Environ. Chem. 8, 225–235.
Tyler, G., 2004. Rare earth elements in soil and plant systems - a review. Plant Soil 267, 191–206.
Van Gestel, C.A.M., Hensbergen, P.J., 1997. Interaction of Cd and Zn toxicity for *folsomia Candida* willem (collembola: isotomidae) in relation to bioavailability in soil. Environ. Toxicol. Chem. 16, 1177–1186.
Van Ginneken, M., De Jonge, M., Bervoets, L., Blust, R., 2015. Uptake and toxicity of Cd, Cu and Pb mixtures in the isopod Asellus aquaticus from waterborne exposure. Sci. Total Environ. 537, 170–179.
Wall, D.H., Nielsen, U.N., Six, J., 2015. Soil biodiversity and human health. Nature 528, 69–76.
Wang, W.X., Yan, Q.L., Fan, W., Yan, X.U., 2002. Bioavailability of sedimentary metals from a contaminated bay. Mar. Ecol. Prog. Ser. 240, 27–38.
Wang, P., Menzies, N., ym, W., Zhou, D.-M., Zhao, F.-J., 2012. Identifying the species of copper that are toxic to plant roots in alkaline nutrient solutions. Plant Soil 361, 317–327.
Wu, M., Wang, X., Jia, Z., De Schamphelaere, K., Ji, D., Li, X., Chen, X., 2017. Modeling acute toxicity of metal mixtures to wheat (*Triticum aestivum* L.) using the biotic ligand model-based toxic units method. Sci. Rep. 7, 9443.
Zaimes, G.G., Hubler, B.J., Wang, S., Khanna, V., 2015. Environmental life cycle perspective on rare earth oxide production. ACS Sustain. Chem. Eng. 3, 237–244.
Zhang, H., Davison, W., Knight, B., McGrath, S., 1998. In situ measurements of solution concentrations and fluxes of trace metals in soils using DGT. Environ. Sci. Technol. 32, 704–710.